Metabolic Regulation: Fasting in the Dark

It is natural to starve. Learning how animals cope by juggling their scarce metabolic resources should therefore allow us to pinpoint physiological switches relevant to diabetes and cardiovascular disease. One known environmental trigger to metabolic adaptation is darkness; new studies suggest its endocrine mediator is 5′ AMP.

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Metabolic regulation is a balancing act operating minute-by-minute and across a lifetime. 5′ AMP, a product of ATP synthesis, is a well-recognised metabolic control point, acting intracellularly via AMP kinase to stimulate fatty acid oxidation, a necessary adaptation when glucose supplies are short [1]. Hibernation and torpor are extreme cases of glucose deprivation, in which glucose is best reserved for the brain, leaving peripheral tissues to get by on stored fat [2]. But how is the switch of energy sources orchestrated [3], and can we exploit these mechanisms to manage metabolic disease?

Working in the dark, Zhang *et al.* [4] have highlighted an intriguing regulatory response by showing that mice exposed to continuous darkness (DD), a putative mimic for the hibernaculum, rapidly switch to fatty acid metabolism, accompanied by increased metabolic gene expression and an elevated daily rhythm of circulating 5′ AMP. The affected genes encode procolipase (mClps) and pancreatic lipase-related protein (mPlrp2), which are known to stimulate fatty acid production, but Zhang *et al.* [4] also found that their expression is controlled by the circadian (daily) clock. Moreover, exogenous 5′ AMP can throw this transcriptional switch and also induce what looks to be torpor in mice under normal light/dark cycles (LD). Is circulating 5′ AMP an agent of darkness (Figure 1)?

Zhang *et al.* [4] enmesh familiar themes, metabolism, 5′ AMP, torpor and circadian clocks into a novel albeit loose weave best unravelled backwards from the endpoint. Mice in DD for a week did not enter torpor but did eat and drink less, lost body weight accordingly and rapidly re-directed metabolic status, as evidenced by lowered blood glucose and increased free fatty acids. This is surprising in laboratory mice, which are not usually photoperiodic and are routinely exposed to DD in circadian studies without reported weight loss or metabolic changes.

Zhang *et al.* [4] might thus have uncovered a previously overlooked phenomenon questioning the neutrality of DD in mice. Nevertheless, these acute responses are entirely consistent with how short daylengths or permanent darkness induce hibernal physiology in photoperiodic species [5], although these natural programmes are typically cued over months not hours. Ectopic up-regulation of lipid metabolising enzymes in tissues where they are not usually expressed, as seen here for mClps and mPlrp, is again well established in hibernators [6], and presumably, calorimetry would confirm the mice were indeed burning different fuels. So, are the mice exercising latent torpor biology or has their response to an acute physiological stress (DD) mimicked some of its components?

The second surprise is that, by high-performance liquid chromatography, Zhang *et al.* [4] tracked down circulating 5′ AMP as a potential cue: levels were found to be increased both in DD conditions and in mice rendered torpid by food deprivation. Importantly, exogenous 5′ AMP triggered both mClps expression and torpor in mice under LD conditions. Torpor, however, occurred rapidly and was completed before mClps expression increased and blood glucose fell. So is suppression of core body temperature the principal action of 5′ AMP and the metabolic adjustments a consequent stress response, or are these responses evidence of a co-ordinated programme of 5′ AMP actions?

The sequential circadian peaks of endogenous 5′ AMP and expression of mClps and mPlrp2 under DD conditions, and the loss of rhythmic gene expression in circadian mutants, provide temporal correlations suggestive of a physiological programme. It would therefore be important to know whether exogenous 5′ AMP

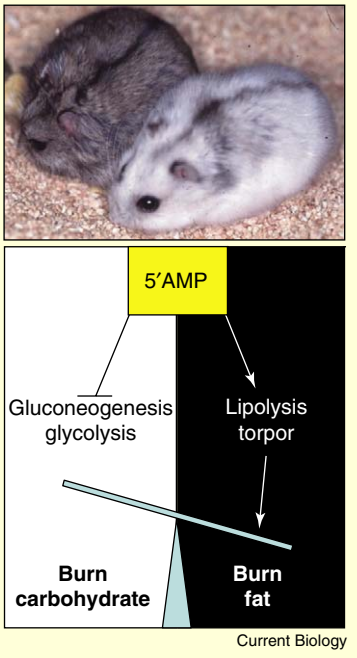

Figure 1. Natural hibernators and species that show torpor, such as Siberian hamsters (*Phodous sungorus*), conserve energy and re-direct metabolic fuels.

In the top panel, the animal on the right shows white winter pelage triggered by lengthening nights. It will also burn more fat and less carbohydrate than its summer (brown) counterpart. Short-term studies in mice now suggest that darkness can activate a 5′ AMP-dependent signalling cascade that drives metabolic adaptation to a 'winter' condition of torpor and fat mobilisation. (Photograph courtesy of F.J.P. Ebling.)

can restore rhythmic gene expression in clock mutant mice, thereby establishing potential causal mechanisms, and whether rhythmic expression of these genes facilitates metabolic adaptation. Intriguingly, circadian deficient mice were found to be more sensitive to the torpor inducing actions of 5′ AMP, although this was evident before any acute changes in gene expression could have been completed. The level(s) at which the body clock impacts on this metabolic regulation is therefore unclear — is it the 5′ AMP signal, the reactive metabolic genes or both?

How might darkness act in these mice? If it were simply due to the absence of ocular stimulation, one would expect elevated 5′ AMP and the putative metabolic shift in variously blind mice. Similarly, we know that retinopathy occurs in diabetes, but conversely, can blindness in humans bias metabolic fuel usage away from glucose towards fatty acids? Downstream from the retina, the pineal hormone melatonin is released at night under circadian control, and is an extremely well characterised physiological mediator of darkness and night-length [7]. Zhang *et al.* [4], however, used C57Bl6 mice, which are melatonin-deficient because they have a mutated gene for serotonin N-acetyltransferase [8]. Conventional melatonin-dependent photoperiodic signalling is therefore unable to account for the metabolic responses of these mice, so is the circadian clockwork involved in other ways?

Any circadian biologist will tell you that nocturnal activity bouts in photophobic rodents expand on transfer from LD to DD [9], and in human athletes, musculo-skeletal activity can alter 5′ AMP dependent physiology. Increased locomotion in the mice may therefore have raised 5′ AMP levels, altering local and systemic gene expression and metabolic fuel usage. Moreover, activity cycles are disrupted in circadian mutant mice thereby dysregulating their response to DD. Unfortunately, Zhang *et al.* [4] do not present any activity records, and since such behavioural responses to DD are completed within a couple of weeks, it would be useful to know whether the metabolic changes observed by Zhang *et al.* [4] were transient, or persisted with a time course comparable to that of hibernation.

What are the broader issues raised by this study? The first is the complex effect of environmental light on physiology. Zhang *et al.* [4] report that gene expression under DD reverses five hours after re-exposure to light. This may reflect a change in rest/activity behaviour, but equally we know that light can act via the circadian pacemaker of the suprachiasmatic nuclei (SCN) and the autonomic nervous system to rapidly alter gene expression in peripheral organs [10]. The psychologically arousing effects of light are well documented and it is also possible that light affects our metabolism via subliminal neural pathways.

Second, it is clear that metabolism is under circadian control. Tissue-based clocks control the local circadian transcriptome, and are in turn synchronised to each other and to solar time by behavioural and neuroendocrine cues emanating from the SCN [11]. The particular case of circadian expression of 5′ AMP, mPlrp2 and mClps raises the general issue of how the circadian mechanism directs adaptive, temporal segregation of incompatible and competitive metabolic pathways. Disruption to circadian timing, either by mutation in mice [12] or by shiftwork in people [13], is associated with metabolic disease. Where 5′ AMP sits in this process remains to be determined, but Zhang *et al.* [4] have provided a further tantalising example of how temporal organisation underpins normal physiology. Armed with growing epidemiological, physiological and molecular genetic evidence, the challenge for the circadian community is not only to show that bad timing creates poor health, but also to provide practical solutions to sustain society in its 24/7 madness.

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